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Genetics

The human protooncogene product p33pim is expressed during fetal hematopoiesis and in diverse leukemias

(cancer genetics/embryogenesis)

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ABSTRACT **We measured the human *pim-1* protooncogene (*PIM*) expression during fetal development and in hematopoietic malignancies. Our data indicate that during human fetal hematopoiesis the 33-kDa *pim* product, p33pim, is highly expressed in the liver and spleen. In contrast, at the adult stage it is only slightly expressed in circulating granulocytes. Out of 70 hematopoietic malignancies analyzed, 51 patients and 19 cell lines, p33pim was overexpressed in ≈30% of the samples, particularly in myeloid and lymphoid acute leukemias. This overexpression was unrelated to any stage of cellular differentiation and was not due to gene rearrangement or amplification. These results imply a physiological role of the *pim-1* protooncogene during hematopoietic development and a deregulation in various leukemias.**

The human *pim-1* protooncogene (*PIM*) is localized on chromosome 6p21 (1), a fragile site involved in particular leukemias (2). The RNA transcript is 2.9 kilobases (kb) long and the cDNA contains an open reading frame of 313 codons (3–5). The protein product is a 33-kDa cytoplasmic tyrosine kinase (6), which we refer to as p33pim. The human protein is homologous to the mouse *pim-1* product, showing 94% identity in amino acid sequence. In the mouse this gene was found to be activated by proviral insertion in murine leukemia virus-induced T-cell lymphoma (7, 8). Such insertions resulted in elevated expression of the *pim-1* mRNA or its truncated forms (9). It was found in other systems that proviral insertion can result in activation of oncogenes such as c-*myc* in avian leukosis (10), c-*erb* in erythroblastosis (11), and c-*myb* in plasmacytoid sarcomas (12). It was therefore anticipated that *pim-1* could be a transforming gene, and this was recently demonstrated in transgenic mice (13). Moreover, a cooperation between *pim-1* and the *myc*-family genes in lymphomagenesis was suggested.

The present arguments supporting the involvement of *pim-1* in human malignant transformation are based upon the analysis of the RNA in various cell lines, in which it is occasionally overexpressed (5). Some of the mechanisms (14) responsible for the overexpression of human protooncogenes in tumors are gene amplifications, rearrangements, and mutations in control elements. On the other hand, oncogenes can be overexpressed either in a specific cell type and stage during fetal development or in the process of physiological cellular growth and differentiation (14, 15).

Experiments on the conservation of oncogenes through phylogeny and their expression in development were of major importance to define their role (15, 16). Initial studies on the c-*abl* and c-*fos* oncogenes have already implicated them in mouse development (17). The *int-1* oncogene, which is also activated by insertion of proviral DNA, was shown to be expressed in the developing mouse nervous system and in adult mice in postmeiotic spermatids. Recently this gene was found to be the homologue of the *Drosophila* segment polarity gene wingless (18). As far as human oncogenes are concerned, such developmental studies are lacking because of the difficulty in obtaining embryonic and fetal tissues. Limitations encountered in some previous studies on oncogenes in human tumors were due to the fact that conclusions were drawn from observations on cell lines, which do not always faithfully represent the original neoplastic clone, and on a limited number of well-characterized patients. Moreover, most of those studies were carried out at the level of RNA, which for some genes such as c-*fos* (19) and *myc* (20) has a short half-life and may not faithfully represent the expression of the protein.

To understand the role of an oncogene in normal development and to investigate whether its untimely expression contributes to the malignant phenotype, it is important to define the expression of this oncogene at the protein level in fetal tissues and patient tumors. The present study approaches these issues, focusing on *pim-1* expression in the human hematopoietic system.

MATERIALS AND METHODS

Fetal and Adult Tissues, Cell Lines, and Patients. Fetal tissues were processed with informed familial consent within 1 hr and only in cases of spontaneous abortion without prostaglandin administration. One 16-week-old (only liver and spleen), two 18-week-old, and one 24-week-old fetus were analyzed. All the adult tissues were analyzed with informed familial consent less than 1 hr post mortem. The 19 cell lines used have been previously characterized (21, 22). Blasts from patients were isolated as described before (23). Bone marrow cells, polymorphonuclear cells, and mononuclear cells were isolated by standard density gradient centrifugation. Morphologic and immunologic characterization was based on the usual criteria (24–26). Twenty-four acute nonlymphoid (myeloid) leukemias and 27 lymphoid leukemias and tumors were analyzed. Surface antigen phenotyping was performed by using an Epics (Coulter) cytofluorometer with a wide range of antibodies (antibodies in parentheses): CD19 (B4), CD10 (J5), CD20 (B1), CD22 (T015), CD7 (Leu9), CD5 (Leu1), CD2 (OKT11), CD1 (OKT6), CD4 (Leu3a), CD8 (OKT8), CD3 (Leu4), CD25 (Tac), CD33 (My9), CD13 (My7), CD14 (My4), CD15 (ION1), CD11b (OKM1), CD42 (Sz2),

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Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoid leukemia.

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CD41 (Sz21), CD38 (OKT10), (OKT9), platelet glycoproteins Ib and IIb/IIIa, and major histocompatibility complex class II molecules DR, DP, and DQ.

Constructs, Antibodies, Immunoprecipitation, and Western Blotting. The TrpE-pim expression plasmid was prepared by ligating the 209-base-pair *Bgl* II–*Acc* I fragment of c-*pim* 7 clone into the *Bam*HI–*Cla* I site of the pATH2 vector (6). Expression of the fused TrpE-pim protein and preparation of the antibodies has been described (6). The same purified TrpE-pim was used to generate monoclonal antibodies by following standard procedures, and screening was performed as described before (27). Two monoclonal antibodies (mp1 and mp2) were prepared.

Immunoprecipitation was performed by following the previous protocol (6), except that for immunoprecipitation with the monoclonal antibodies, rabbit antibodies to mouse immunoglobulins were added 30 min before the addition of staphylococcal protein A-Sepharose. Specific inhibition of immunoprecipitation with the fused TrpE-pim was performed as described (6). Western blot analysis was done according to standard procedures (28). To 5×10^6 cells 50 μl of $NaDodSO_4$ sample buffer was added and the proteins were separated by $NaDodSO_4$/10% polyacrylamide gel electrophoresis. After electrophoresis proteins were transferred to a nitrocellulose membrane for 16 hr at 70 V, 4°C. Monoclonal anti-pim antibodies were used in a dilution of 1:500. As a secondary reagent, ^{125}I-labeled donkey antibodies to mouse immunoglobulins (Amersham) were used. Tissues were homogenized in a Dounce homogenizer (4°C), and 5 mg of the original tissue sample was resuspended in 1 ml of $NaDodSO_4$ sample buffer. After boiling and centrifugation at $14,000 \times g$, 10 μl was loaded on the gel (corresponding to 50 μg of protein). Blots were stained with Ponceau red (Sigma) and the autoradiographs were scanned with an LKB Ultroscan densitometer.

The Western blots were rehybridized with monoclonal antibody Ab-1 (Oncogene Science, Mineola, NY), which is specific for human c-myc, at a dilution of 1:100.

The effect of cycloheximide on p33pim expression was evaluated by incubating K562 cells in the presence of cycloheximide at 10 μg/ml at 37°C for various time intervals. Also, the half-life of the protein was evaluated by labeling K562 cells with [^{35}S]methionine for 1 hr followed by a chase and immunoprecipitation at various time intervals.

The initial identification of the pim protein by using monoclonal antibodies mp1 and mp2, was carried out on the human hematopoietic cell line K562, which was previously shown to contain high levels of *pim-1* mRNA and to express the gene product (3, 6). Western blot analysis (Fig. 1*A*) of K562 extracts indicates that both antibodies recognize specifically the 33-kDa pim protein. This was further analyzed by immunoprecipitation (Fig. 1*B*) of [^{35}S]methionine-labeled K562 cells and confirms our previous results with the polyclonal anti-pim antiserum (6).

Immunohistochemistry. Fetal liver was fixed overnight in 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.5, at 4°C. Sections 20 μm thick (Vibratome Lancer) were washed in 0.01 M Tris·HCl-buffered saline, pH 7.5 (TBS), and incubated in 2% (vol/vol) H_2O_2/TBS for 15 min. The sections were then incubated in TBS containing 0.1% Triton X-100 for 30 min, followed by 60 min at room temperature with normal goat serum (1:10), and then anti-pim antibodies (1:250) or anti-TrpE antibodies (1:250) were added for an overnight incubation (4°C). After three washes in TBS containing 0.1% Triton X-100 the secondary antibody (goat antibody to rabbit immunoglobulins) was added for 90 min at room temperature and the immunoperoxidase reaction was performed.

For histologic analysis the sections were stained with hematoxylin/eosin.

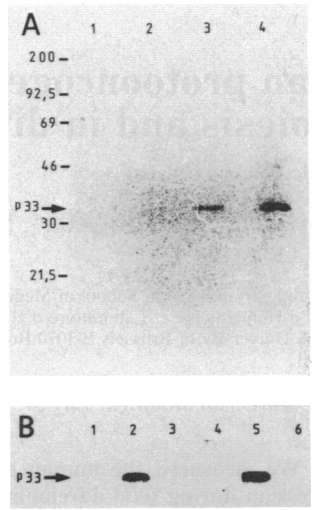

FIG. 1. Identification of the human p33pim by monoclonal antibodies. (*A*) Five million cells from the erythroleukemic cell line K562 were lysed in $NaDodSO_4$ sample buffer and the proteins were separated by $NaDodSO_4$/10% PAGE. The proteins were transferred to nitrocellulose and Western blot analysis was performed with various primary antibodies and with ^{125}I-labeled donkey antibody to mouse immunoglobulins (Amersham) as secondary antibody. Lanes: 1, secondary antibody alone; 2, normal mouse serum; 3, monoclonal anti-pim antibody mp1; 4, monoclonal anti-pim antibody mp2. Arrow indicates the position of p33pim. The molecular mass markers indicated on the left are ^{14}C-labeled Rainbow standards (Amersham). (*B*) Immunoprecipitation of pim-1 protein from metabolically [^{35}S]methionine-labeled K562 cells. Lanes: 1, preimmune rabbit serum; 2, anti-pim polyclonal antiserum; 3, anti-pim polyclonal antiserum in the presence of the fused TrpE-pim protein; 4, normal mouse serum; 5, monoclonal anti-pim antibody mp1; 6, monoclonal anti-pim antibody mp1 in the presence of the fused TrpE-pim protein.

Southern Blotting. The DNA of all 24 acute myeloid leukemia (AML) patients was analyzed. Restriction enzyme analysis was performed with *Eco*RI and *Bam*HI or *Hin*dIII. The *Bgl* II–*Eco*RI fragment of clone c-*pim* 7 was used as 5′ probe and the *Hin*dIII/*Eco*RI fragment from c-*pim* 9 was used as 3′ probe (3). As control for the amount of DNA loaded, a C_β (generous gift of J. L. Strominger; Harvard University, Cambridge, MA) or J_γ probe (generous gift of T. H. Rabbitts; (Medical Research Council, Cambridge, U.K.) was used as described (23).

RESULTS

Developmental Regulation of the *pim-1* Gene Product in Hematopoiesis. The implication of the *pim* oncogene in murine hematopoietic malignancies suggests that, like some other oncogenes, it may play a physiological role in embryonal and fetal development. We investigated *pim* expression during normal human fetal hematopoiesis. Four cases with appropriate tissue preservation were selected.

The *pim* oncogene was highly expressed in the fetal liver and spleen of the 16- and 18-week-old fetuses. In the 24-week-old fetus expression was still elevated in the liver but decreased in the spleen. On the other hand, *pim* was not expressed in the corresponding adult tissues (Fig. 2*A*). At the adult stage a weak expression was found only in the polymorphonuclear cell fraction. Upon overexposure of the autoradiographs a faint band corresponding to p33pim was found in the kidney of the 18-week-old fetus and also in the adult bone marrow (data not shown). To further analyze the cells expressing *pim* in fetal liver, an immunohistochemical analysis was performed. Fig. 2 *B–D* shows that p33pim was

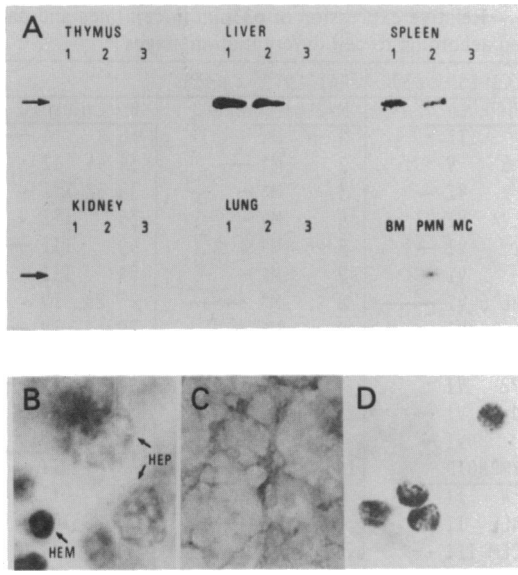

FIG. 2. Expression of human p33pim in fetal and adult hematopoiesis. (*A*) Western blot analysis of various tissues with monoclonal antibody mp2 in an 18-week-old fetus (lanes 1), 24-week-old fetus (lanes 2), and adult (lanes 3). BM, adult bone marrow; PMN, adult polymorphonuclear fraction; MC, adult mononuclear cell fraction. Arrow indicates the position of p33pim. (*B*) Section of 18-week-old fetal liver stained with hematoxylin/eosin. The intensively stained small round cell is a hematopoietic progenitor (HEM); it is surrounded by large cells that are the hepatocytes (HEP). (×300.) (*C* and *D*) Immunohistochemical analysis of 18-week fetal liver with anti-TrpE (*C*) and anti-pim (*D*) antibodies revealed by immunoperoxidase reaction. (×300.) The four cells in *D* reactive with the anti-pim antibody are small round hematopoietic progenitors.

specifically expressed in typical round cells corresponding to islands of hematopoiesis in the fetal liver.

Expression of p33pim in Hematopoietic Cell Lines and Leukemia. To investigate the potential implications of the *pim* oncogene in human malignant transformation, we analyzed its expression in hematopoietic cell lines and patient tumors at different stages of differentiation. Screening by Western blotting with monoclonal antibody mp2 (Fig. 3) of 19 cell lines of myeloid, myelomonocytic, and B- and T-lymphoid origin at different stages of differentiation indicated variable levels of p33pim expression. The highest amounts were found in the K562 and KCL cells (myeloid origin) and in SUDHL-6 cells (histiocytic origin). For the other cell lines the amount of p33pim was lowest in T-cell lines (HPB ALL, MOLT-4, CEM) and myelomonocytic cells (e.g., HL60). These results suggest that p33pim is expressed in hematopoietic and lymphoid tumors. This expression could be related to myeloid differentiation or particular stages of lymphoid maturation.

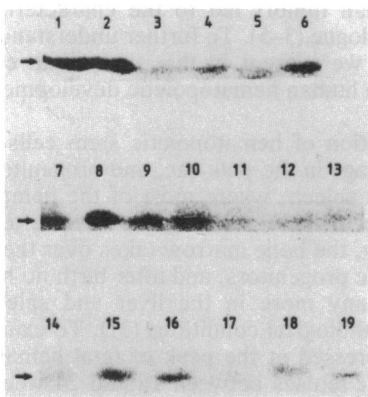

FIG. 3. Expression of p33pim in cell lines. Western blot analysis of hematopoietic cell lines with monoclonal anti-pim antibody mp2. Cell lines were as follows: 1, K562 (erythroleukemia); 2, KCL (myeloid); 3, HPB ALL (T-lymphoid; ALL = acute lymphoid leukemia); 4, HP1 (B hairy cell); 5, CEM (T-lymphoid); 6, Jok 1 (B hairy cell); 7, SB (B-lymphoid); 8, SUDHL-6 (histiocytic lymphoma); 9, IM9 (B-lymphoid); 10, ARH77 (B-lymphoid); 11, Molt-4 (T-lymphoid); 12, UC729 (B-lymphoid); 13, Nalm 6 (non-B non-T); 14, KM3 (B-lymphoid); 15, Daudi (Burkitt lymphoma); 16, Raji (Burkitt lymphoma); 17, HL60 (myelomonocytic); 18, U937 (myelomonocytic); 19, NAMALVA (Burkitt lymphoma). Arrow indicates p33pim.

The sequence analysis (3) indicated that the 3′ noncoding region of *pim-1* contains multiple A-U motifs, suggesting an unstable mRNA. We have investigated the turnover of p33pim. In the presence of cycloheximide (Fig. 4*A*) K562 cells do not express detectable levels of p33pim after 15 min of incubation. From metabolically labeled K562 cells (Fig. 4*B*) no labeled p33pim was immunoprecipitated after a chase of 15 min. These data indicate that pim is a protein with a short half-life, ≈10 min.

Since the above findings relate to cell lines, some of them derived from the original clone more than 10 years ago, we decided to analyze also p33pim expression in fresh tumor cells from a large number of patients with leukemia. The analysis of 24 patients with AML (Fig. 5*A*) indicates that *pim-1* overexpression is found at various stages, including M0 (patient 22), M1 (patient 8), M2 (patient 10), and M5 (patient 20). In the analysis of 27 patients with lymphoid malignancies (Fig. 5*B*) overexpression was found in one B-lineage ALL, stage IV (patient 29), and one completely immature ALL (patient 38). These data indicate that in human leukemia *pim-1* overexpression is not correlated with a particular cell type or differentiation stage (summarized in Table 1). It is therefore likely that *pim-1* overexpression results from an inappropriate activation in various leukemias. Southern blot analysis of DNA from all the AML patients indicates that

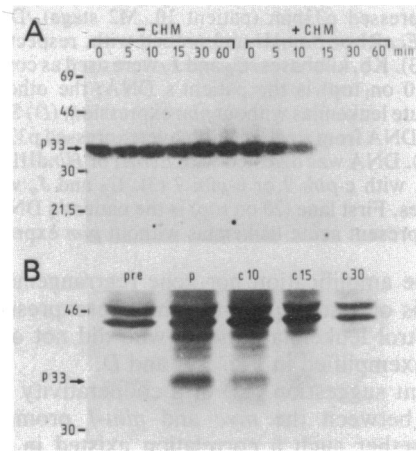

FIG. 4. Turnover of p33pim in K562 cells. (*A*) The stability of p33pim was investigated by incubating K562 cells in the absence (− CHM) or presence (+ CHM) of cycloheximide. After incubation for the indicated times, the samples were analyzed on Western blots, using monoclonal anti-pim antibody mp2. (*B*) The half-life of p33pim was evaluated by labeling K562 cells with [^{35}S]methionine (pulse of 1 hr) followed by a chase with medium containing unlabeled methionine at various times. The proteins were extracted and the immunoprecipitation reaction was carried out. Lane 1 (pre): cells labeled with a 1-hr pulse of [^{35}S]methionine and extracts immunoprecipitated with preimmune normal mouse serum. Lane 2 (p): cells labeled with a 1-hr pulse of [^{35}S]methionine and extracts immunoprecipitated with monoclonal anti-pim antibody mp1. Lanes 3–5 (c10–c30): cells labeled with a 1-hr pulse of [^{35}S]methionine followed by a chase of, respectively, 10 min (c10), 15 min (c15), or 30 min (c30), and extracts immunoprecipitated with monoclonal anti-pim antibody mp1.

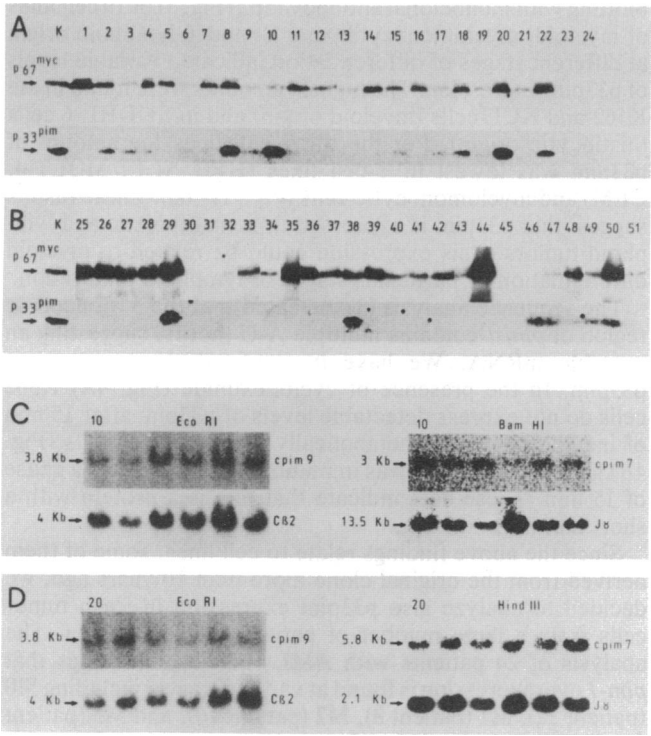

FIG. 5. Expression of p33pim and analysis of the *pim-1* gene in hematopoietic malignancies. (*A*) Western blot analysis of p33pim in 24 patients with AML (for classification see Table 1) using monoclonal anti-pim antibody mp2. K, control cell line K562. The lower band, with the arrow labeled $p33^{pim}$, represents the position of the *pim-1* gene product. In a second step the same Western blots were hybridized with the monoclonal anti-c-myc antibody (upper band, with arrow labeled $p67^{myc}$). (*B*) Western blot analysis of p33pim in 27 patients with ALL (lanes 25–51) using antibody mp2. Bands are as described for *A*. (*C*) Southern blot analysis of DNA from a patient with overexpressed p33pim (patient 10, M2 stage). DNA was digested with *Eco*RI or *Bam*HI and probed with, respectively, c-*pim* 9 or c-*pim* 7 (3). Kb, kilobases. C_β and J_γ were used as control probes. First lane (10 on top) is the patient's DNA; the other five lanes represent acute leukemias without *pim* expression. (*D*) Southern blot analysis of a DNA from a patient with overexpressed p33pim (patient 20, M5 stage). DNA was digested with *Eco*RI or *Hin*dIII and probed, respectively, with c-*pim* 9 or c-*pim* 7 (3). C_β and J_γ were used as control probes. First lane (20 on top) is the patient's DNA; the other five lanes represent acute leukemias without *pim* expression.

neither gene amplification nor gene rearrangement occurs. The analysis of 2 such patients who overexpressed p33pim and 10 control leukemia patients who did not overexpress p33pim is exemplified in Fig. 5 *C* and *D*.

The recent suggestion (13) of a cooperativity in lymphomagenesis between the *myc* and *pim-1* prompted us to analyze whether such a correlation existed in the human leukemia samples analyzed here. Western blot analysis with anti-c-myc antibodies of cells from the 51 patients with leukemia indicated no consistent correlation between c-*myc* and *pim-1* expression (Fig. 5 *A* and *B*). Indeed, while in some cases both p33pim and p67myc are overexpressed (e.g., patients 29 and 38), in other cases either p33pim (e.g., patient 10) or p67myc (e.g., patient 44) can independently be overexpressed.

DISCUSSION

Growing evidence suggests the involvement of protooncogenes in malignant transformation. To achieve cell transformation it seems that interaction between two or more oncogenes (29) or a lack of interaction between an oncogene and a suppressor gene is of crucial importance (30). The activation of a transforming gene may occur as a result of point mutation, chromosomal translocation, insertional mutation, amplification, or deregulation (14).

Table 1. Relative expression of p33pim in cell lines and patients classified according to cell differentiation stages

CELL LINES	p33	PATIENTS	p33		
B LYMPHOID		MYELOID		B LYMPHOID	
KM3	III •	1	M1 •	38	I ——
NALM 6	V •	2	M1 ——	34 44	II •
RAJI	VI —	3	M1 –	30 32	III •
NAMALVA	VI -	4	M1 -	35	III •
DAUDI	VI —	5 6	M1 •	49	III —
SB	VI —	7	M1 -	51	III -
SU DHL 6	VI ——	8	M1 ——	25 26	IV •
IM 9	VI —	9	M2 –	29	IV ——
ARH 77	VI —	10	M2 ——	33	IV •
UC 972	VI •	11 12	M2 •	47	IV —
JOK 1	VI —	13 14	M3 •	36	BL •
HP 1	VI –	15	M4 –	T LYMPHOID	
T LYMPHOID		16	M4 •	43	I •
MOLT 4	II •	17	M5 –	48	II –
HPB ALL	II -	18	M5 ——	27 28	III •
CCRFCEM	III -	19	M5 –	31 45	III •
NON LYMPHOID		20	M5 ——	46	III ——
K562	——	21	M5 •	50	III ——
KCL	——	22	M0 ——	37	CLT •
U937	-	23 24	M0 •	39 40	SL •
HL 60	•			41 42	SL •

Numbers 1–51 correspond to patients in Fig. 5 *A* and *B*; M0–M5 denote the French–American–British classification (25); and roman numerals correspond to T- or B-cell differentiation stage (24). BL, B-cell lymphoma; CLT, T-cell chronic leukemia; SL, Sezary lymphoma. Dots and lengths of bars are relative to the densitometric measures of the autoradiographs (Figs. 3 and 5 *A* and *B*).

Different systems have been developed to study such genes. One of those is exemplified by formation of lymphomas in mice by murine leukemia viruses. Indeed, the insertion of those retroviruses, devoided of "onc" sequences, at a very particular locus (e.g., *pim-1*) results in formation of T-cell lymphomas (7). The potential involvement of the *pim-1* gene in human tumors led to the characterization of the human homologue (3–5). To further understand the function of this gene we present in this study data concerning its expression in human hematopoietic development and malignancies.

The formation of hematopoietic stem cells starts at the embryonal stage in the yolk sac, and progenitors migrate to the liver and spleen, where most of the hematopoietic development occurs between 16 and 24 weeks after conception. Progressively, the bone marrow takes over the formation of hematopoietic progenitors, and after birth no hematopoiesis takes place any more in the liver and spleen except in particular pathological conditions (31). To examine whether *pim* was expressed at the peak of fetal hematopoiesis, we analyzed four fetuses between 16 and 24 weeks after conception. Our data indicate that p33pim is expressed in the fetal liver and spleen. More specifically, by using immunohistochemistry, we detected p33pim in the liver in typical hematopoietic progenitors and not in the surrounding tissues. In contrast, at the adult stage the expression of *pim* in the liver and spleen is shut off except for minute amounts in the polymorphonuclear cell fraction. Many of the oncogenes have been implicated in development, but because of the difficulty in obtaining embryonal tissues, knowledge remains limited and most information is derived from murine fetal development, where c-*fos*, c-*fms*, and c-*myb* are expressed during hematopoiesis (15). In humans, such studies can be

exemplified by the overexpression of c-*myc* in a stage- and cell-specific pattern, mostly in the proliferative germinal cells of skin and gut epithelia (32), and by *src* expression in the brain (33).

The expression of *pim* during normal hematopoietic development and its subsequent shut-off in the adult raise the possibility that its untimely overexpression at the adult stage may contribute to malignant transformation. Our results on p33pim expression in acute leukemias suggest that this gene is deregulated in various stages of cell differentiation. In K562 cells p33pim has a short half-life, ≈10 min. This overexpression is not due to gene amplification. Such mechanisms of overexpression without amplification were previously reported for oncogenes such as c-*myc* in leukemias (34) and N-*myc* in Wilms tumors (35). Particularly for c-*myc*, a broad study (36) of primary cells from human acute leukemias indicates that this gene is expressed in all cases of AML, at variable levels and without correlation with the French–American–British classification. The same study also indicates that c-*myc* is widely expressed in ALL, with an increased level in pre-B lymphocytes. Since transgenic mice bearing *pim-1* show a predisposition to lymphomagenesis with a suggestive cooperativity in transformation between *pim-1* and the *myc* family genes (13), we tested the expression of c-*myc* in the 51 patients. As in previous studies, c-*myc* was widely expressed in over 90% of the patients, and we found no obvious parallelism between c-*myc* expression and *pim-1* expression. This indicates that both *pim-1* and the c-*myc* may cooperate independently with other oncogenes in transformation. However, our results do not exclude the possibility that in cases in which both genes are overexpressed, an effective cooperation may exist.

Cytogenetic analysis of the leukemic cells from the 51 patients did not show any structural abnormality at the short arm of chromosome 6, although in four cases a trisomy (patients 2, 33, 42, and 49) or even a tetrasomy (patients 29 and 39) of chromosome 6 was observed. Southern blot analysis indicated no rearrangement within the gene. The above results also indicate that *pim-1* is not involved in myeloid or lymphoid differentiation, since *pim* overexpression was not correlated with a particular cell stage or cell type. However, the possibility remains that during induction of a differentiation process *pim* may be down- or upregulated. *In vitro* differentiation of K562 cells to megakaryocytes induced by addition of phorbol 12-tetradecanoate 13-acetate shows that *pim* mRNA and protein levels drop after 24–48 hr and reach 30–50% of their initial value after 4 days of induction. Nevertheless, control experiments suggest that this may be due to growth arrest rather than to the differentiation induction itself (R.A., A.T., R. Alitalo, and K. Alitalo, unpublished observation).

In conclusion, our data show that p33pim is expressed mainly during embryonal development in tissues involved in hematopoiesis. In addition, it is overexpressed in some adult leukemias, independently of cell stage and cell type. Such untimely overexpression of the *pim-1* oncogene may contribute to the malignant phenotype. However, as for other oncogenes expressed without any correlation with differentiation or proliferative state of the tumor cells and with no detectable amplification or gene rearrangement, the precise changes underlying this overexpression remain to be elucidated. The possibility exists that in the various tumor cells expressing the *pim-1* a common mechanism of activation may operate.

Investigations involving the transcriptional control and posttranscriptional events will eventually provide a better insight into the mechanisms of *pim-1* overexpression.

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